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Delignification of Oil Palm Frond via particle size reduction and chemical pretreatment for enhanced biogas production potential

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Abstract

Oil palm frond (OPF) is an abundant lignocellulosic biomass with significant potential as a renewable energy source. However, its high lignin content limits its biodegradability and efficiency in biogas production. This study examined the effects of particle size reduction and chemical pretreatment (using NaOH/HCl mixtures) on lignin removal and structural modification of OPF. Particle sizes of 0–250 µm, 251–500 µm, and 501–750 µm were pretreated at 150–190°C for 4–12 minutes, with NaOH/HCl ratios between 0.001 and 0.1. The pretreatment reduced lignin content from 22.6%, 24.7%, and 25.5% in raw OPF to 9%, 12%, and 13%, respectively. Fourier Transform Infrared (FTIR) analysis indicated the reduction of amine-hydroxy ketone bands (from 1636 to 1625 cm^{-1}), while Scanning Electron Microscope (SEM) images revealed surface stripping and disorganization of lignocellulosic fibres, improving cellulose accessibility. The proximate composition and volatile solids showed minimal change, confirming substrate suitability for biogas conversion. These findings demonstrate that combined particle size reduction and alkaline-acid pretreatment effectively enhance delignification and bioconversion potential of OPF for sustainable bioenergy production.

1.0 Introduction

Agricultural residues are increasingly recognized as valuable feedstocks for renewable energy production. In Nigeria, oil palm fronds (OPF), corn stover, rice husk, and sugarcane bagasse are generated in large quantities, yet most are discarded or burnt (Oshoro *et al.*, 2024). The wastes are mostly produced from the Pisifera and Dura species of oil palm trees available in Nigeria. The Dura has large nuts with thick shells and thin mesocarp, while the Pisifera has a tiny fruit without a shell. Stavila *et al*. (2023) reported that the Dura specie's availability in the Southern parts of Nigeria, produces significant amount(s) of the wastes.

The palm wastes comprise of cellulose, hemicellulose, lignin, water-soluble compounds, and fibres, called lignocellulose wastes (Rodionova *et al.* (2022). The lignocellulose part of the plant is called the plant's dry part, known as Ligno-Cellulosic Biomass (LCB). LCB has gained much attention in research, due to its renewable nature. It is one of the most promising natural feedstocks, that can be obtained from renewable sources to produce energy, agrochemicals, modern industry materials, and animal feed. Most LCBs contain 40-50 % cellulose, 25-35 % hemicellulose, and 15-27 % lignin (Mirmohamadsadeghi *et al*., 2021). The distribution of the constituents can vary significantly amongst different types of plants (Mirmohamadsadeghi *et al*., 2021). Cellulose, hemicellulose, and lignin, which form the significant constituents of biomass, depend on the age, environment, soil condition, and weather effect of the plants (Mirmohamadsadeghi *et al*., 2021).

The Oil palm frond, a lignocellulosic material composed primarily of cellulose, hemicellulose, and lignin, represents an underutilized biomass source. The high lignin content, however, hinders microbial degradation and reduces biogas yield during anaerobic digestion (Sai-Bharadwaj *et al*., 2023). Lignin forms a complex matrix with cellulose and hemicellulose, acting as a physical barrier that limits enzymatic and microbial accessibility to structural carbohydrates during anaerobic digestion (Hendriks & Zeeman, 2009; Karrupiah and Azariah, 2019; Taherzadeh & Karimi, 2008). However, complete lignin removal can also degrade cellulose and hemicellulose, emphasizing the need for optimized delignification methods (Oyegoke *et al*., 2020). Various pretreatment techniques like mechanical, thermal, and chemical approaches have been employed to improve the digestibility of lignocellulosic substrates. Ball milling, for instance, reduces particle size and partially disrupts the lignin–cellulose structure (Karrupiah and Azariah, 2019), while acid or alkaline pretreatments solubilize lignin and hemicellulose (Karrupiah and Azariah, 2019). However, no single pretreatment method has achieved complete delignification without compromising carbohydrate content (Karrupiah and Azariah, 2019).

Few studies have examined the synergistic effect of combining microwave-assisted NaOH/HCl pretreatment with fine particle size optimization, for oil palm frond delignification, under low chemical loading conditions, even though particle size reduction and alkaline pretreatment have been studied separately, for lignocellulosic biomass conversion. Recent developments in physicochemical pretreatment have shown that combination pretreatment techniques frequently enhance biomass digestibility and lignin disruption more successfully than single pretreatment techniques (Banu Jamaldheen *et al*., 2022; Mankar *et al*., 2021). Therefore, this study introduces a pretreatment method combining particle size reduction with dual NaOH/HCl chemistry under microwave heating, to enhance lignin solubilization, preserve cellulose, and accelerate reaction kinetics. Three OPF particle sizes were assessed to determine their impact on delignification, using low chemical concentrations (0.001–0.1 M) to minimize environmental effects. Structural changes were evaluated using FTIR, SEM, proximate analysis, and volatile solids, with the overall aim of determining how this combined pretreatment improves lignin removal, structural modification, and biogas production potential of OPF.

2.0 Materials and Methods

2.1 Sample Collection and Preparation

Oil palm fronds (OPF) were collected from freshly pruned oil palm trees in Kantigi Forest, Edati Local Government Area, Niger State, Nigeria (latitude 9.31371°N, longitude 6.10143°E). The collected biomass was thoroughly washed with tap water, followed by distilled water, to remove adhering soil and impurities. The samples were air-dried for several days, and subsequently oven-dried at 65°C, until constant weight was achieved. The dried OPF samples were milled and sieved into three particle size fractions: 0–250 μm, 251–500 μm, and 501–750 μm, using standard laboratory sieves and a mechanical sieve shaker operated for 15 minutes, to ensure uniform particle separation. Reducing the particle size increases the available surface area of the biomass and improves the accessibility of cellulose microfibrils to chemical pretreatment and microbial degradation during anaerobic digestion.

2.2 Lignin Removal from Oil Palm Fronds

Hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions with concentrations ranging from 0.001 M to 0.1 M were used for the pretreatment of oil palm fronds (OPF). The OPF samples were prepared in three particle size ranges: 0–250 μm, 251–500 μm, and 501–750 μm.

For each experiment, HCl and NaOH solutions were mixed at an acid-to-base volumetric ratio of 4:1. A total of 10 g of OPF was added to 50 mL of the prepared reagent mixture in a 100 mL beaker. The mixture was then heated in a microwave reactor at temperatures between 150 and 190°C for 4–12 minutes. Temperature control was achieved using the built-in temperature monitoring system of the microwave reactor. Microwave heating was selected because it provides rapid and uniform heating of polar biomass components, which enhances lignin breakdown and improves pretreatment efficiency. Temperatures above 190°C

were avoided to prevent cellulose degradation and carbonization, while reaction times longer than 12 minutes were avoided to minimize sugar degradation. The 4:1 acid–base ratio was selected to combine mild acid hydrolysis of hemicellulose with alkaline lignin solubilization, thereby promoting effective and selective delignification.

After pretreatment, the solid biomass was separated by filtration and washed several times with distilled water until a neutral pH was obtained. The treated biomass was then dried in an oven at 65°C for 72 hours.

All experiments were conducted in triplicate to ensure reproducibility and reliability of the results. The average values obtained from the experimental runs were reported, and standard deviations were calculated, where applicable.

2.3 Characterization of the Raw and the Pretreated OPF substrate

2.3.1 The volatile and proximate composition

The volatile solids and proximate composition of the untreated and pretreated oil palm fronds (OPF) were determined, to evaluate the effect of pretreatment on the physicochemical characteristics and biodegradability of the biomass substrate. The analyses include the determination of moisture content, volatile solids, ash content, crude fibre, crude protein, crude fat, and carbohydrate content, using standard analytical procedures described by Rosario *et al*. 2021. All analyses were conducted in triplicate to ensure reproducibility and reliability of the experimental results.

The samples were analyzed before and after pretreatment, to assess the changes in biomass composition, resulting from the chemical pretreatment process. Standard laboratory equipment and controlled experimental conditions were used throughout the analyses to minimize experimental error.

2.3.2 Fourier Transform Infrared Analysis

Fourier Transform Infrared (FT-IR) analysis was used to investigate the functional groups of the organic compounds present in the raw and pretreated OPF substrate; the Agilent FTIR 8400s model (from the manufacturer: Malvern Panalytical) was used for the analysis. Bands were recorded as transmittance functions within the wave number range 4000 - 450 cm-1 at a resolution of 10 cm-1. Vibrations of OH, C-H, C=O, C=C, C=O, C-O, and C-O groups, where the band peaks at the wave number of approximately 3630 cm-1 and 3500-3200, 1715, 1647, 1515, 1247, 1364, 1032, and 895 cm-1, were investigated.

2.3.3 Scanning Electron Microscope (SEM) Analysis

Phenom ProX desktop model (from the manufacturer: Benchtop) SEM was used to analyse the morphological features of the raw and pretreated OPF sample, at particle sizes of 0-250 µm, 251-500 µm and 501-750 µm. The sample powder was sprinkled on a thin layer primary fixation with aldehyde (proteins), and a tape was placed on the brass sample holder. The adhered sample was layered with ethanol or acetone, followed by drying, mounting on stub, and sputter coating with conductive material.

3.0 Results and Discussions

3.1 Composition of Oil Palm Fronds

Table 1 Composition of raw oil palm fronds (OPF)

Particle size of the sample (µm)	**Lignin** %	Hemicellulose %	**Cellulose** %	**Reducing Sugar** %
0-250	22.65 ±0.45	26.5 ±0.5	42.5 ±0.5	2.7 ±0.2
251-500	24.65 ±0.35	25.0 ±0.4	37.5 ±0.5	1.9 ±0.3
501-750	25.5 ±0.5	24.5 ±0.5	36.0 ±1.0	1.3 ±0.1

The lignocellulose composition of the Oil palm fronds cell walls, which include cellulose, hemicellulose, lignin and reducing sugar contents, was determined for different particle sizes (0-250 µm, 251-500 µm and 501-750 µm), as shown in Table 1. The table shows that all the errors in the lignin removal, cellulose yield, hemicellulose yield and reducing sugar yields, in the particle size(s) of 0-250 µm, 251–500 µm and 501–750 µm, were less than one percent, which can be considered to be insignificant. The major constituents shown in Table 1 are cellulose, followed by hemicelluloses, lignin, and a negligible amount of reducing sugar. The composition depends on particle size, as shown in the table, and presented by Muhammad (2024). The lignin composition decreases with decrease in particle size; this agrees with literature (Muhammad, 2024), which suggests that there is destruction of the lignin seal, which may enhance cellulose and hemicellulose matrixes in the ball milling process, which is likely responsible for the reduction in lignin composition. The highest amount of cellulose, hemicellulose, and reducing sugar were found in the smallest OPF particle size of 0-250 µm, which agrees with literature (Ali *et al*., 2024), which noted that fine milling improves cellulose exposure and facilitates microbial digestion. The finest particle size (0–250 µm) exhibited lower lignin and higher cellulose due to increased disruption of lignin–carbohydrate complexes during milling. Reduction in particle size increases specific surface area and reduces mass transfer limitations, thereby improving penetration of pretreatment chemicals into the lignocellulosic matrix (Izumi *et al*., 2010). Again, this agrees with observations from previous studies (Ali *et al*., 2024; Muhammad 2024) that mechanical reduction enhances carbohydrate exposure.

3.2 Effect of particle size and pretreatment on Lignin removal

Apart from particle size reduction, microwave irradiation was deployed to raise the pretreatment temperature while the NaOH/HCl (ratio) and pretreatment time were also varied, in order to investigate their effect(s) on the composition of lignin. Alkaline pretreatment selectively removes lignin, while preserving carbohydrate fractions and increasing biomass

porosity (Kim *et al.*, 2016). NaOH catalyses cleavage of lignin ether and ester linkages, such as β–O–4 bonds, promoting lignin solubilization and increasing cellulose accessibility. HCl mildly hydrolyses hemicellulose and enhances cell wall swelling. While microwave heating intensifies these reactions by creating localized hotspots and micro-explosions within the biomass matrix, increasing surface disruption and lignin removal efficiency (Jasmine *et al.*, 2023; Venegas-Vásconez *et al.*, 2025). Thus, the combined effects of these variables were observed.

For particle size(s) of 0-250 µm, 251-500 µm and 501-750 µm, and ranges of pretreatment conditions of NaOH/HCl ratio 0.001-0.1, temperature 150-190ºC, time 4-12 minutes, pretreatment was carried out on the OPF, with the results shown in Figure 1. The figure shows that lignin yield decreased, with increase in NaOH/HCl ratio, temperature and time, and decrease in particle size. Following the pretreatment(s), the percentage(s) of lignin were reduced to 9%, 12% and 13%, for particle size(s) of 0 - 250 µm, 251 - 500 µm and 501 - 750 µm respectively. The lignin removal was 50.1% out of the 22.6% lignin present in the 0 - 250 µm particle size, 48.4% out of the 24.1% in the 251-500 µm particle size, and 48.2% of the 25.5% in the 501-750 µm particle size. The lowest lignin (of 9%) was achieved at a particle size of 0-250 µm, under the conditions of NaOH/HCl ratio of 0.1, temperature of 190ºC and time of 12 minutes; this residual lignin content is below the threshold of 15% lignin content, which implies that the pretreated sample is suitable for optimal biogas production (Soheil *et al.*, 2017). Therefore, Lignin removal improved with increased NaOH/HCl ratios, temperature, and time, and with reduced particle size. Maximum delignification (9% residual lignin) occurred at 0–250 µm particle size, 190°C, 12 minutes, and NaOH/HCl ratio 0.1.

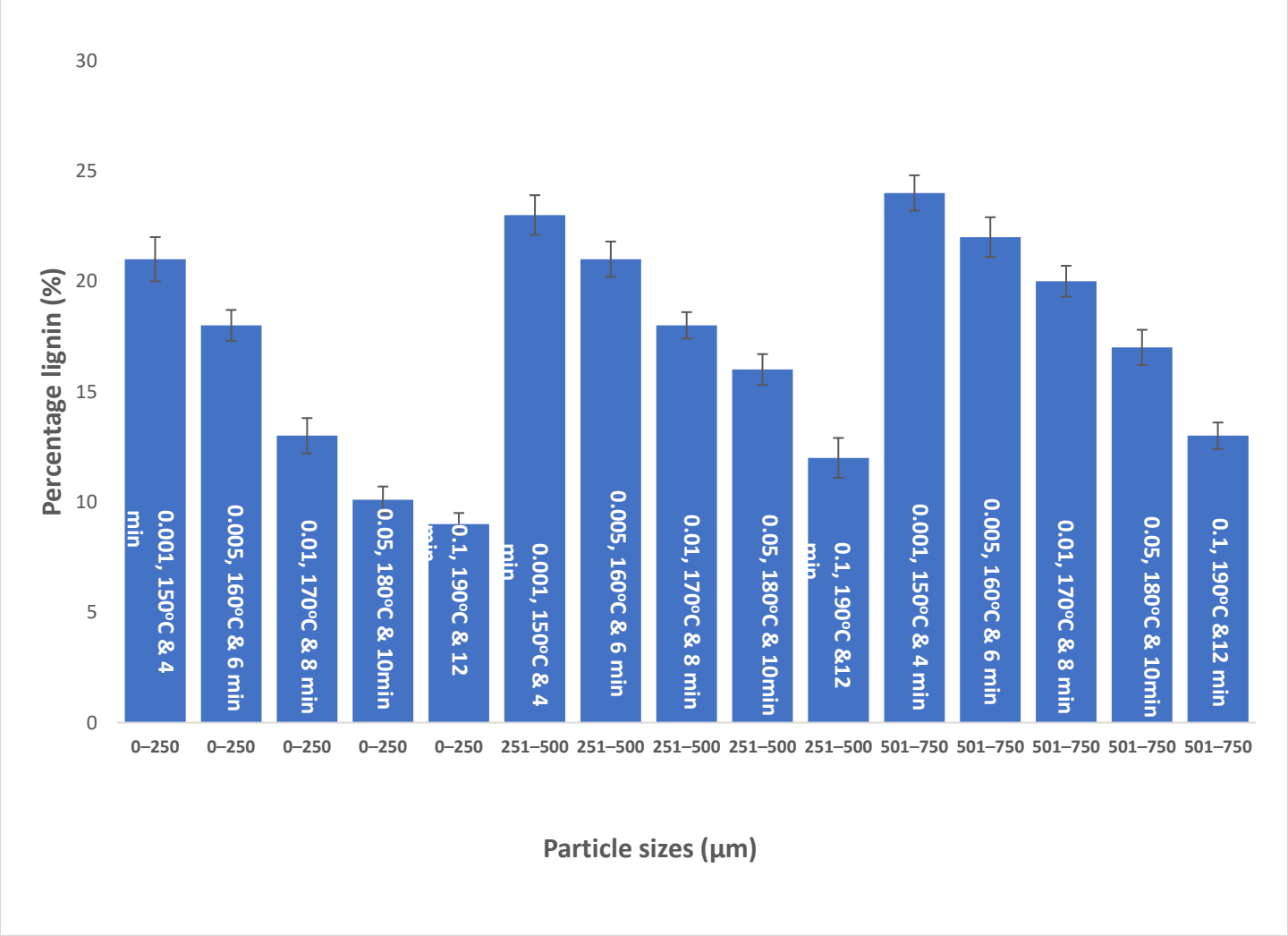

Figure 1 Effect of NaOH/HCl ratio, microwave temperature, pretreatment duration, and particle size on residual lignin content of oil palm frond

3.3 Effect of pretreatment conditions on OPF Constituents

Table 2 The delignification at different particle sizes

Particle Sizes (µm)	NaOH/ HCl) ratio	Temperature (°C)	Time (Min)	Cellulose (%)	Hemicellulose (%)	Reducing Sugar (%)
0-250	0.1	190	12	38.0±0.5	25.8±0.1	1.9±0.1
251-500	0.1	190	12	35.5±0.4	23.1±0.3	1.7±0.2
501-750	0.1	190	12	35.1±0.3	23.1±0.2	1.1±0.1

Table 2 shows the effect of pretreatment on the other constituents of the OPF. When compared with the raw constituents (Table 1), it can be seen that the constituents were reduced to 39.7% lignin, 89.4% cellulose, 97.4% hemicellulose, 70.4% reducing sugar of the initial amount(s) for particle size of 0-250 µm; 48.7% lignin, 94.7 cellulose, 92.4% hemicellulose, 89.5% reducing sugar for particle size of 251-50 µm; and 51% lignin, 97.5 cellulose, 94.3% hemicellulose, and 84.6% reducing sugar for particle size of 501-750 µm, after the pretreatment. The yields show that the feedstock is viable for biogas production. Jon *et al.* (2020) reported that crystalline cellulosic structure shielding the cellulose can be removed during pretreatment, and the cellulose remains can be easily converted to biogas by anaerobic bacteria. Also, Jon *et al*. (2020) reported that the pretreatment of the substrate before the biogas production could increase the digestibility of the lignocellulosic materials, raise the biodegradation rate, and improve biogas yield in biological energy conversion processes. Therefore, carbohydrate loss was minimal, with cellulose and hemicellulose retaining 89–97% of their original values, across particle sizes. This selective lignin removal is critical for maintaining biogas production potential. This is especially important, since delignification improves microbial accessibility to fermentable polysaccharides during hydrolysis, which is generally considered the rate-limiting stage of anaerobic digestion of lignocellulosic biomass (Hendriks & Zeeman, 2009; Taherzadeh & Karimi, 2008).

3.4 Chemical and Structural Analysis

3.4.1. Volatile solid of the OPF

Table 3 Volatile solid and Proximate analysis of OPF

Properties %	Raw OPF	Pretreated OPF (0 - 250µm)	Pretreated OPF (251 - 500µm)	Pretreated OPF (501 - 750µm)
Volatile solid	81.9±1.2	81.8±2.3	81.7±3.0	81.7±1.5
Moisture content	95.5±1.5	95.3±1.2	95.3±2.0	95.3±1.8
Ash	13.5±1.0	13.4±1.1	13.5±1.4	13.5±1.3
Protein	13.9±2.0	13.9±1.2	13.9±1.3	13.9±1.3
Lipid	9.9±1.3	8.9±1.1	8.9±1.2	8.9±1.2
Fiber	21.9±1.4	20.1±1.2	21.1±1.3	21.2±1.1
Carbohydrate	53.6±1.7	50.2±1.4	50.2±1.4	50.2±1.6

The proximate composition of the oil palm fronds before pretreatment include: moisture content of 95.5% ± 1.5, ash 13.5% ± 1.0, protein 13.9% ± 2.0, lipid 9.9% ± 1.3, fibre 21.9% ± 1.4, and carbohydrate 53.6% ± 1.7. Similarly, the pretreated OPF substrate at particle size 0–250 µm recorded moisture content of 95.3% ± 1.5, ash 13.4% ± 1.1, protein 13.9% ± 1.2, lipid 8.9% ± 1.2, fibre 20.1% ± 1.2, and carbohydrate 50.2% ± 1.4, indicating that there was no significant difference between the proximate composition of the raw and pretreated OPF substrates. Comparable proximate compositions were also observed for the other particle sizes (251–500 µm and 501–750 µm), as presented in Table 3. The proximate composition and volatile solids (81.7–81.9%) displayed minimal variation before and after pretreatment, demonstrating that the chemical treatment did not compromise the overall organic matter available for anaerobic digestion (Carlos and Paula, 2024). This suggests that the delignification process selectively targets lignin without causing substantial loss of fermentable carbohydrates.

The relatively high carbohydrate content observed after pretreatment is advantageous for anaerobic digestion because carbohydrates constitute the primary readily biodegradable fraction responsible for rapid microbial hydrolysis and methane generation. Increased carbohydrate availability suggests that pretreatment enhanced exposure of fermentable polysaccharides by partially disrupting lignin, thereby improving substrate digestibility and biogas production potential (Taherzadeh & Karimi, 2008). Similarly, the preservation of cellulose after pretreatment is desirable because cellulose is the major fermentable polysaccharide that contributes to methane formation during anaerobic digestion. Increased cellulose accessibility improves enzymatic hydrolysis efficiency and substrate conversion (Sun & Cheng, 2002). Hemicellulose, being relatively more amorphous and easier to hydrolyze than cellulose, may also contribute positively to rapid microbial degradation and volatile fatty acid production during anaerobic digestion when moderately preserved after pretreatment (Mosier, 2005).

The high volatile solids content (observed in Table 3) indicates substantial organic biodegradable matter within the biomass. Volatile solids are directly associated with the amount of organic substrate available for microbial conversion during anaerobic digestion; therefore, increased volatile solids content generally corresponds to improved methane generation potential (Li *et al*., 2011). Furthermore, the relatively low ash content observed after pretreatment is beneficial because excessive ash represents

inorganic non-biodegradable material that may reduce the effective organic fraction available for methane production. High ash concentrations can also negatively affect digester performance through mineral accumulation (Kumar *et al.*, 2015).

Reduction in lignin content following pretreatment is particularly beneficial because lignin is highly recalcitrant and resistant to microbial degradation under anaerobic conditions. Lower lignin concentration enhances accessibility of cellulose and hemicellulose to hydrolytic microorganisms, thereby improving biodegradability and methane yield potential (Hendriks & Zeeman, 2009). Overall, the minimal changes observed in the proximate composition after pretreatment, indicate that the treatment strategy effectively disrupted lignin, while preserving the biodegradable carbohydrate-rich fractions essential for efficient biogas production.

3.4.2. Fourier transform infrared spectroscopy.

Table 4 The Infrared Band Frequencies of the functional groups present in the OPF

Infrared Band Range (cm^{-1})	**Common functional group in the band range**	**Band Peaks (cm^{-1})**	**Components**
3500-3200	O-H (H-bonded)	3287.5	Cellulose
2840-2690	C-H (Aldehyde)	2918.5	Cellulose
1745-1720	Halogenated derivatives	1733.3	C= in Xylan (hemicellulose)
1680-1620	Amide	1636	Lignin skeletal in vibration
	Hydroxy ketones	1625	C-H deformation
	C-H		
1600-1500	Conjugated cyclic	1543	C=O, C=C Conjugated lignin
	C=O, C=C	1513.3	Deformed C=O, C=C
1448-1330	C-H	1408.9	
		1364.2 & 1367.9 1315.8	Deformed C-H in lignin
1270-1000	C-O	1233.7	Syingyl Lignin and co-stretch lignin
		1028.7	Deformation in cellulose & hemicellulose
1000-400	C-H	757.3	C-H deformation in cellulose
		663.5	C-H deformation in cellulose

FTIR was used to identify the functional groups in the OPF substrates, before and after pretreatment. The result is summarized and shown in Figure 2 and Table 4. Table 4 shows the summary of the infrared band range, common functional groups assigned, band peaks, and the components assigned to the functional groups. The analysis of Table 4 shows that cellulose has band peaks of 3287.5 cm-1 and 2918.5 cm-1, which were reduced to 1636.5 cm-1, 1028.7 cm-1, and 757.3 cm-1. The reduction of the peaks showed that cellulose has been strongly enhanced during particle size reduction and pretreatment. While hemicellulose has a peak of 1733.3 cm-1. The lignin peaks are 1636 cm-1, 1543 cm-1, and 1408.9 cm-1, which were reduced to 1367.9 cm-1, 1364.2 cm-1, 1315.8 cm-1, and 1233.7 cm-1, known as deformed lignin, C=C, C=O and C-H components respectively. The reduction in the band peaks of lignin, during particle size reduction and pretreatment, denote reduction of lignin in the pretreated OPF substrate. Reduction of absorption bands around 1510–1630 cm^{-1} is commonly associated with aromatic skeletal vibration loss and lignin degradation during pretreatment (Xu *et al.*, 2013).

Comparing the particle sizes of 0-250 µm and 251-500 µm, it shows that the same functional groups, such as O-H, C-H, hydroxy ketones and conjugated cyclic are present in both OPFs. This implies that reducing the particle size of the substrate from 251-500 µm to 0-250 µm, does not seem to have a significant effect on the functional groups of the OPF substrates. Similarly, the FTIR spectra for particle sizes of raw OPF of 500-750 µm and 0-250 µm, showed similar functional groups.

Finally, FTIR spectra revealed reduced peak intensities for amine-hydroxy ketones (from 1636 to 1625 cm^{-1}), signifying lignin degradation. The retention of O–H and C–H stretching bands indicates that cellulose and hemicellulose structures remained largely intact, which is similar to the work reported by Latika & Dilipkuma (2023). Hence, significant reductions in lignin-associated bands (1636 → 1625 cm^{-1} and 1543 → 1513 cm^{-1}) indicate aromatic ring degradation and ether bond cleavage. Meanwhile, cellulose peaks (3287 and 2918 cm^{-1}) remained dominant, confirming structural stability.

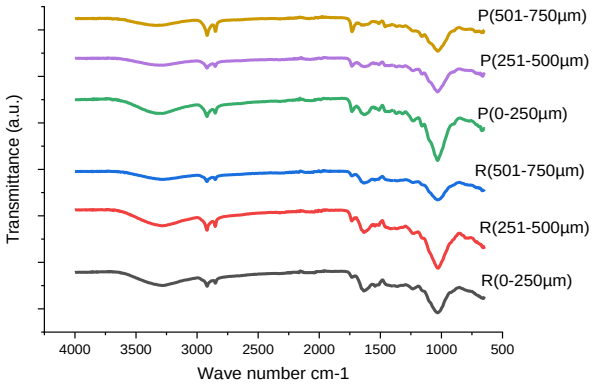

Figure 2 Superimposed FTIR SPECTRA for (a) Raw 'R' OPF, and (b) Pretreated 'P' OPF, for the different particle sizes.

3.4.3. Scanning Electron Microscopy

The changes in the structures and surface area of the raw and pretreated OPF were analysed via Scanning Electron Microscopy (SEM). SEM images of the raw OPF are shown in Plates a1, b1, and c1, while the pretreated OPF images are shown in Plates a2, b2, and c2. The structure of the raw OPF displayed a rigid, organized and highly ordered microfibril structure of the OPF, compared to the pretreated OPF. In particular, the structures of the pretreated OPF are distorted and crumpled in the particle size of 0-250 µm. In the pretreated OPF substrate with particle size of 251-500 µm, the topmost surface was stripped off, while the 501-750 µm particle size showed broken microfibril after the pretreatments. The study has identified that the pretreatment of the OPF caused an abrasion of the surface morphology, which is lignin. The topmost structures were more affected because of the direct contact with the conditions of pretreatment. The initially smooth and organized topmost surface of the raw OPF was severely damaged, as the surface became disfigured and loosened, after pretreatment. The pretreatment also destroyed the internal cell structure, generating some irregular cracks, as shown in Plates a2, b2 and c2. Structural rupture, pore formation, and fibre disorganization observed after pretreatment, are characteristic indicators of effective lignocellulosic disruption and enhanced substrate accessibility (Kumar *et al*., 2009). Similarly, Latika & Dilipkuma (2023) showed that rice husk and substrate structure, became disorganized after the materials were pretreated. Thus, the lignin in the pretreated OPF was degraded, increasing the exposure of cellulose and hemicelluloses in the substrate to microbial attack.

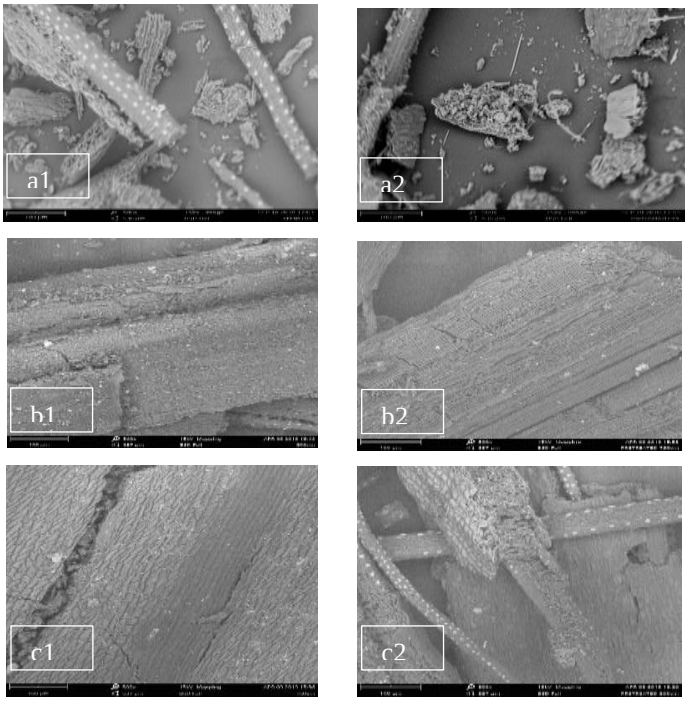

Plate 1 SEM of 500 Magnification for Raw OPF of size: (a1) 0-250 µm, (b1) 251-500 µm, (c1) 501-750 µm, and SEM of pretreated OPF of size (a2) 0-250 µm (b2) 251-500 µm, (c2) 501-750 µm

3.5 Comparison with the Previous Studies

In comparison with earlier pretreatment studies, conventional methods typically report lignin removals of about 25–35% for steam explosion pretreatment, 30–40% for dilute acid pretreatment, 35–45% for simple NaOH (alkali) pretreatment, and only 15–25% for biological pretreatments (Bhushan *et al*., 2023; Chen *et al*., 2024), while the combined microwave and dual chemical approach used in this study achieved up to 60% lignin removal, clearly exceeding these values. Consequently, untreated oil palm residues, which commonly yield about 150–190 L CH_4 per kg VS (Qian *et al*., 2025), are projected to produce about 200–260 L CH_4 per kg VS after this treatment, due to the high lignin removal and the preservation of carbohydrate fractions, demonstrating a substantial improvement in methane generation potential.

4.0 Conclusions

The raw OPF substrate at particle sizes of 0-250 µm, 251-500 µm, and 501-750 µm contain 22.65%, 24.65%, and 25.5% lignin, respectively. For the pretreatment conditions of NaOH/HCl ratio of 0.1, a temperature of 190ºC, and a time of 12 minutes, at the particle sizes of 0-250 µm, 251-500 µm, and 501-750 µm, the lignin was reduced to 9%, 12%, and 13%, respectively. Therefore, particle size reduction and pretreatment conditions affect the lignin content. The volatile solid and proximate composition did not show any significant differences between the raw and pretreated OPF. While FTIR analysis showed that the band peaks of lignin have been reduced to 1367.9 cm-1, 1364.2 cm-1, 1315.8 cm-1, and 1233.7 cm-1 and SEM analyses confirmed structural disruption and exposure of fibrous networks, essential for microbial attack during biogas production. The negligible change in proximate and volatile solid composition, further validates OPF's suitability as a biogas feedstock. Therefore, this integrated pretreatment approach offers a pathway for improving bioenergy yields from agricultural residues.

Declaration of conflict of interest

The authors have collectively contributed to the conceptualization, design, and execution of this journal. They have worked on drafting and critically revising the article to include significant intellectual content. This manuscript has not been previously submitted or reviewed by any other journal or publishing platform. Additionally, the authors do not have any affiliation with any organization that has a direct or indirect financial stake in the subject matter discussed in this manuscript.

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